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Whole genome mapping in a wheat doubled haploid population using SSRs and TRAPs and the identification of QTL for agronomic traits

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Abstract Genetic maps are useful for detecting quantitative trait loci (QTL) associated with quantitative traits and for marker-assisted selection (MAS) in breeding. In this research, we used the wheat × maize method to develop a doubled haploid (DH) population derived from the synthetic hexaploid wheat (SHW) line TA4152-60 and the North Dakota hard red spring wheat line ND495. The population consisted of 213 lines, of which a subset of 120 lines was randomly selected and used to construct linkage maps of all 21 chromosomes and for QTL detection. The whole genome maps consisted of 632 markers including 410 SSRs, 218 TRAPs, 1 RFLP, and 3 phenotypic markers, and spanned 3,811.5 cM with an average density of one marker per 6.03 cM. Telomere sequence-based TRAPs allowed us to define the ends of seven linkage groups. Analysis revealed major QTLs associated with the traits of days to heading on chromosomes 5A and 5B, plant height on chromosomes 4D and 5A, and spike characteristics on chromosomes 3D, 4A, 4D, 5A and 5B. The DH population and genetic map will be a useful tool for the identification of disease resistance QTL and agronomically important loci, and will aid in the identification and development of markers for MAS.

Keywords *Triticum aestivum* · Embryo rescue · TRAPs · SSRs · Agronomic trait

Introduction

Many quantitative traits are influenced by the environment, which makes it necessary to perform phenotypic assessments in replicated trials under multiple environments using immortal mapping populations consisting of recombinant inbred lines (RIL) or doubled haploids (DH). RIL populations are developed using the single-seed-descendent (SSD) method, and usually take several years to develop because lines have to be advanced to the $F_{5:6}$ generation or later. In wheat, DH plants can be developed by culturing the anther or microspores (androgenesis) of F_1 plants (Craig 1974), or by pollinating the F_1 floret with maize pollen and embryo rescue to obtain haploid plants, followed by colchicine treatment (Laurie and Bennett 1988). The wheat × maize method is considered to be the more efficient of the two (Kisana et al. 1993). Brazauskas et al. (2004)

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used amplified fragment length polymorphisms (AFLPs) to assay a DH population developed using the wheat × maize method and found no introgression of maize DNA in the wheat genome. A DH mapping population can be developed within about 2 years, and therefore can be generated more rapidly than RIL populations. In addition, DH lines are genetically pure immediately after doubling the chromosome number, whereas RILs usually contain some degree of heterozygosity. One advantage to RILs is that they undergo more rounds of meiotic recombination through the inbreeding process.

PCR-based molecular markers are preferred over other types because of their ease of use. Microsatellite or simple sequence repeats (SSRs) (Beckman and Weber 1992) are the most preferred due to the fact that they are the most user-friendly, highly polymorphic, stable, co-dominant, and usually locus-specific (Akkaya et al. 1992). For whole genome mapping in wheat, obtaining dense linkage maps of all chromosomes requires a large number of PCR reactions when using SSRs as the only marker type. Target region amplified polymorphism (TRAP) (Hu and Vick 2003) is a rapid, relatively high-throughput and efficient marker system, and they can be especially useful when combined with SSRs for developing maps of the whole genome (Liu et al. 2005). Li et al. (2006) developed 302 chromosome-specific TRAP markers to characterize a set of 14 'Langdon' (LDN)-'Chinese Spring' (CS) D-genome chromosome disomic substitution lines, and found that TRAP markers detected polymorphisms in the A, B and D genomes of wheat at nearly equal frequency. Hu (2006) developed nine TRAP fixed primers containing repeat units of *Arabidopsis*-type telomere sequences and generated markers that defined the ends of sunflower linkage groups. One of these telomere sequence-derived fixed primers, TeloR, was also used in wheat by Liu et al. (2005), and six TRAP markers that it detected mapped to the ends of wheat linkage groups. Therefore, telomere sequence-derived TRAP markers likely represent the ends of individual chromosomes, which define the ends of linkage groups and give more accurate estimations of actual genetic lengths.

Previous whole genome mapping endeavors indicated the size of the hexaploid wheat genome in terms of genetic distance ranged from 2,360 to 4,110 cM (Chalmers et al. 2001; Groos et al. 2002; Liu et al. 2005; Paillard et al. 2003; Quarrie et al. 2005; Somers et al. 2004; Song et al. 2005; Sourdille et al. 2003; Torada et al. 2006), but most indicated the wheat genome encompasses about 3,600 cM. However, many of these maps possessed some relatively large gaps, particularly in D-genome chromosomes.

In this study, we developed a DH population derived from the cross between a synthetic hexaploid wheat (SHW) line (TA4152-60) and an elite North Dakota hard red spring wheat line (ND495) using the wheat × maize method, and then constructed linkage maps of all 21 chromosomes using a combination of SSR and TRAP markers, including telomere sequence-derived TRAP markers to cap the ends of linkage groups. The utility of the DH population and the linkage maps were further evaluated by identifying QTLs associated with several important agronomic traits.

Materials and methods

Plant materials

A SHW line (TA4152-60) and an elite hard red spring wheat line (ND495) were used to develop the DH mapping population. TA4152-60 was developed at the International Maize and Wheat Improvement Center (CIMMYT) and provided by the Wheat Genetics and Genomics Resource Center, Kansas State University in Manhattan, KS. This SHW line was derived from a cross between the durum wheat (*T. turgidum* L., $2n = 4x = 28$, AABB genomes) variety 'Scoop 1' and the *Aegilops tauschii* Coss. ($2n = 2x = 14$, DD genomes) accession WPI358 (TA2516). TA4152-60 has resistance to tan spot and Stagonospora nodorum blotch (SNB) (Xu et al. 2004), and our preliminary experiments indicate it also has good levels of resistance to Hessian fly and the fungal diseases leaf rust, Fusarium head blight (FHB), and Septoria tritici blotch (STB) (unpublished data). ND495 is an elite hard red spring wheat line selected from 'Justin*2/3/ND 259/Conley//ND 112', but it is susceptible to the pest and diseases mentioned above (unpublished data). The sweet corn cultivar 'Early Sunglow' was used as a pollen source to induce haploid wheat embryos. The wheat Chinese Spring (CS) nullisomic-tetrasomic (NT) lines (Sears 1954, 1966), where a pair of missing chromosomes is partially compensated by an extra pair of homoeologous chromosomes, were used for assigning

markers to individual chromosomes after the linkage maps were constructed.

DH population development

The DH population was developed using the method described by Matzk and Mahn (1994). Briefly, sweet corn was sown ten days prior to the F_1 derived from TA4152-60 × ND495. During the heading stage, about 90 spikes (20 florets per head) from 15 F_1 plants were emasculated and pollinated with sweet corn pollen 4–5 days later. Twenty-four hours following pollination, spikes were sprayed with 2,4-D solution (213.05 mg/l, pH = 10.36), and the spikes were collected for embryo rescue 14–16 days later. Around 20% of florets had haploid embryos, and a total of 394 embryos were rescued and placed in 25 mm diameter tubes containing MS medium with sucrose and agar (pH = 5.8) (Sigma-Aldrich Inc., St. Louis, MO) at room temperature with about 10 h light per day, which finally yielded 261 haploid plantlets. As the haploid plantlets reached 5–7 cm, they were transferred to pots with soil in the greenhouse. At the tillering stage, the plants were removed from the pots and placed in a beaker containing 1,000 ml colchicine solution [colchicine (0.5g/l) + DMSO (20 ml/l) + GA_3 (100 mg/l) + Tween 80 (0.3 ml/l)] at room temperature in the dark for 8 h, and then rinsed with flowing water overnight and transferred back to soil. A total of 228 plantlets remained alive after colchicine treatment, and 213 of them were fertile and used to form the DH population. A subset of 120 DH lines was then randomly selected and used for genetic mapping and QTL analysis.

Marker generation

Total genomic DNA was isolated using the method described by Faris et al. (2000). The DNA concentration was adjusted to 30–50 ng/μl for SSR and TRAP PCR reactions. For SSR markers, 746 SSR primer sets including 333 BARC (Song et al. 2005), 252 GWM (Röder et al. 1998a, b), 28 GDM (Pestsova et al. 2000), 62 CFA (Sourdille et al. 2003), 30 CFD (Guyomarc'h et al. 2002), 39 WMC (Somers et al. 2004) and 2 FCP (Reddy L and Faris JD unpublished) were used to screen the parents for polymorphism. Based on surveys of previously published physical (Sourdille et al. 2004) and genetic (Somers et al. 2004) maps, we selected 10–15 evenly distributed polymorphic markers per chromosome for mapping in the DH population.

PCR reactions for SSR markers were performed as described by Röder et al. (1998b). Electrophoresis was carried out on 6% polyacrylamide gels (0.4 mm thick) in 1 × TBE (90 mM Tris–borate, 2 mM EDTA) at 60 W for 2 h. Gels were stained using 0.001% GelRed (Biotium, Inc., Hayward, CA) for 20 min and scanned using a Typhoon 9410 variable mode imager (GE Healthcare, Inc. Waukesha, WI).

TRAP marker analysis was conducted as described by Hu and Vick (2003). A total of 17 fixed primers were used (Table 1), of which five (W01, W09, W11, W13 and W15) were used by Liu et al. (2005), five (W09, W10, W14, W22 and W55) by Li et al. (2006), and the two telomere-sequence based primers (TeloR and TeloTRG) were used and described in Hu (2006). The remaining primers were newly designed from mapped wheat EST sequences (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi) using the web-based program PRIMER3 (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi) (Rozen and Skaletsky 2000) as described in Xu et al. (2003). Two arbitrary primers, T03 (5′-CGTAGCGCGTCAATT ATG, 5′-end labeled with 700-IR) and T13 (5′-GCGCGATGATAAATTATC, 5′-end labeled with 800-IR), were described by Xu et al. (2003). TRAP markers were given the designation of *Xfcp* in accordance with the reserved laboratory designations of J. D. Faris (http://wheat.pw.usda.gov/ggpages/Lab.Designators.html).

In addition, the RFLP probe *FCG28* was placed on the linkage group for chromosome 5A. *FCG28* is a fragment of the *Q* gene, which was cloned by Simons et al. (2006). Procedures for RFLP analysis were performed as described in Faris et al. (2000). Three phenotypic markers including glaucousness and reaction to the fungal host-selective toxins Ptr ToxA and SnTox1 were also placed on the linkage maps. Glaucousness is a waxy layer that confers a glaucous appearance or white bloom on the wheat leaf and stem surfaces. The glaucousness gene *W1* was located on chromosome 2BS and the dominant inhibitor gene *Iw2* was located on 2DS (Liu et al. 2007). ND495 showed the glaucous phenotype and TA4152-60 was non-glaucous. The presence/absence of glaucousness was recorded for each DH line at the ear emergence stage in the greenhouse.

Table 1 List of the designation and sequence of the fixed primers, source EST accession numbers and the designation of TRAP markers mapped in TA4152-60 × ND495 DH population

Fixed primer[a]	Fixed primer sequence	Wheat EST accession no. (chromosome location)	TRAP marker designations
W01	ATCCATCATCTCCAGAGC	BE637568 (1AS, 1BS, 1DS)	*Xfcp400–Xfcp407*
W03	GGAGATACCTGCCATCAT	BE406450 (1AS, 1BS, 1DS)	*Xfcp408–Xfcp421*
W07	GATGATCGAGGAGAAGGA	BE406918 (1AL, 1BL, 1DL)	*Xfcp422–Xfcp439*
W09	TATCGTCACTTACGCCAG	BE425566 (2AL, 2BL, 2DL)	*Xfcp440–Xfcp448*
W10	CGTTCCTCAAGTGGTACA	BE638034 (2AL, 2BL, 2DL)	*Xfcp449–Xfcp452*
W11	GAAACTTCCAGTTACCCG	BE426431 (2AS, 2BS, 2DS)	*Xfcp453–Xfcp465*
W13	GGTGAAAGAGTTTCCGAC	BE406551 (3AL, 3BL, 3DL)	*Xfcp466–Xfcp480*
W14	CCTCTTGACAAAGGAAGC	BG606778 (3AL, 3BL, 3DL)	*Xfcp481–Xfcp499*
W15	GGAGGATCATGACCAGTT	BE426356 (3AS, 3BS, 3DS)	*Xfcp500–Xfcp520*
W16	CATCAGTTCGACTAGGCA	BE637127 (3AS, 3BS, 3DS)	*Xfcp521–Xfcp539*
W22	GCTGACCTTCCATTGAGT	BE500894 (5AL, 5BL, 5DL)	*Xfcp540–Xfcp553*
W26	ACTCAACAGGCTTGTCCT	BE422631 (6AL, 6BL, 6DL)	*Xfcp554–Xfcp569*
W43	GGCATTATCCACTGTCCT	BE426317 (4AL, 4BL, 4DL)	*Xfcp570–Xfcp574*
W51	GAATTCAGCTTCACGGAC	BE405227 (6AS, 6BS, 6DS)	*Xfcp575–Xfcp586*
W55	GCTTCCCTACAACAAACC	BE405234 (7AS, 7BS, 7DS)	*Xfcp587–Xfcp600*
TeloR	AACCCTAAACCCTAAACC	–	*Xfcp601–Xfcp607*
TeloTRG	CCCAAAACCCAAAACCCAAAAG	–	*Xfcp608–Xfcp617*

[a] W01, W09, W11, W13 and W15 were used by Liu et al. (2005); W09, W10, W14, W22 and W55 were used by Li et al. (2006); TeloR and TeloTRG were two telomere sequence-based fixed primers that were used by Hu (2006); the remaining six fixed primers were designed for this project

Ptr ToxA and SnTox1 are host selective toxins (HSTs) produced by the wheat tan spot (*Pyrenophora tritici-repentis*) and SNB (*Stagonospora nodorum*) pathogens, respectively. Sensitivity to Ptr ToxA is conferred by the *Tsn1* gene on chromosome arm 5BL (Faris et al. 1996). Sensitivity to SnTox1 is controlled by the *Snn1* locus on 1BS (Liu et al. 2004). We used purified Ptr ToxA (Zhang et al. 1997) and partially purified SnTox1 (Liu et al. 2004) (both provided by S.W. Meinhardt, Department of Plant Pathology, North Dakota State University, Fargo, ND) to infiltrate leaves of the DH lines at the two-leaf stage as described in Xu et al. (2004). Plants were scored 4 days after infiltration as sensitive or insensitive based on the presence or absence of necrosis.

Linkage map construction

The segregation ratios of all markers were tested using the computer program QGENE (Nelson 1997) for fit to the expected 1:1 ratio by χ^2 analysis. The computer program MAPMAKER (V2.0) for Macintosh (Lander et al. 1987) was used to perform linkage analysis with a minimum LOD threshold of 3.0 and the Kosambi mapping function (Kosambi 1944). Linkage groups were first identified using the ''two-point/group'' command with a minimum LOD = 3.0 and a maximum $\theta = 0.40$, which yielded several very large linkage groups. Each large group was then regrouped using a minimum of LOD = 10.0. The FIRST ORDER and RIPPLE (LOD > 3.0) commands were used to determine the most plausible order of markers within linkage groups. The TRY command was used to add markers that did not RIPPLE at an LOD > 3.0 to the established framework maps. Positions of centromeres on the maps were estimated according to the published physical map (Sourdille et al. 2004).

Trait evaluation

The quantitative traits analyzed include days to heading, plant height, and spike characters (spike length, spikelet number per spike, and spike compactness). All data were collected from multiple experiments. Data for days to heading were collected from one growth chamber experiment, two

greenhouse experiments conducted during January 26–May 20 (experiment 1) and March 12–June 29 (experiment 2) in 2007, respectively, and one field experiment that included two replicates planted in a randomized complete block design (RCBD). In greenhouse experiments, one to three plants per pot were grown and supplemented with artificial light for a 16-h photoperiod. For the growth chamber experiment, two seeds of each line were planted in a supercell cone (Stuewe and Sons, Inc., Corvallis, OR) with sunshine SB100 (Sun Gro Horticulture, Dellevue) and fertilized with Osmocote Plus 15-19-12 (Scotts Sierra Horticultural Product Company, Marysville, OH). Cones were placed in RL98 trays (Stuewe and Sons, Inc., Corvallis, OR) with the temperature set at 21°C and a 14-h photoperiod. The field experiment was conducted in Fargo, ND. Fifteen seeds of each line were sown on May 16, 2007, in a 90 cm-long row with 30 cm of distance between rows. Days to heading were recorded for each plant as the number of days from planting to emergence of the first spike.

Data on plant height were collected from the same greenhouse and field experiments by measuring the plants from the base to the tallest spike at maturity. For spike characteristics, data were collected from the same greenhouse and field experiments using the methods as described in Jantasuriyarat et al. (2004). Briefly, five mature spikes per DH line were randomly selected and measured from the base of the rachis to the tip of the uppermost spikelet, excluding the awns, to determine spike length. The number of spikelets in each spike was counted to determine spikelets per spike, and spike compactness was calculated by dividing the number of spikelets per spike by the spike length.

Statistical and QTL analysis

Bartlett's χ^2 was used to test homogeneity of variances among the different experiments using the SAS program (SAS Institute 1999), and data from homogeneous experiments were combined for QTL analysis. A subset of markers spaced approximately 5–20 cM apart and giving the most complete genome coverage was used for QTL detection. Composite interval-regression mapping was performed using the program Windows QTL Cartographer (v2.5) (Wang et al. 2007) to evaluate marker intervals putatively associated with trait phenotypes. A permutation test with 5,000 permutations was used to determine that the critical LOD threshold of 3.0 in this DH population yields an experiment-wise significance level of 0.05. Markers with the most significant effect for each QTL for a given trait were assembled into multiple regression models using the computer program QGENE (Nelson 1997). The coefficient of determination (R^2) from the multiple regression model is the total amount of variation explained by the markers.

Results

SSR and TRAP marker generation

Among 746 SSR primer pairs screened for polymorphism between TA4152-60 and ND495, 452 (60%) detected polymorphic fragments. Of these, 268 primer pairs including 117 BARC, 101 GWM, 23 CFA, 12 GDM, 10 WMC, 3 CFD, and 2 FCP were selected for mapping in the DH population and detected 449 SSR marker loci. For the identification of TRAP markers, 18 fixed primers in combination with two random primers (36 primer combinations) were used to directly screen the DH population without prior testing of the parents for polymorphism. A total of 261 TRAP markers were identified, with an average of about 7–8 markers per primer combination. The two telomere-sequence-based fixed primers in combination with different random primers yielded 17 markers.

Linkage map construction

A total of 714 markers (449 SSRs, 261 TRAPs, 1 RFLP and 3 phenotypic markers) were obtained. Eighty-two markers that were either unlinked or formed small linkage groups that could not be assigned to chromosomes were eliminated from the data set, leaving 632 markers (410 SSRs, 218 TRAPs, 1 RFLP and 3 phenotypic markers), which were used to assemble the 21 linkage groups (Fig. 1, Table 2). For anchoring linkage groups to specific chromosomes, 78 of the 266 SSR primer pairs were tested on the whole set of CS NT lines, which allowed us to assign 111 SSR loci to individual chromosome with 2–8 loci per chromosome (Fig. 1). The total genetic distance of the linkage maps was 3,811.5 cM, with an average density of one marker per 6.03 cM.

The map lengths were 1,326.1, 1,243.3, and 1,242.1 cM for the A, B and D genomes, respectively

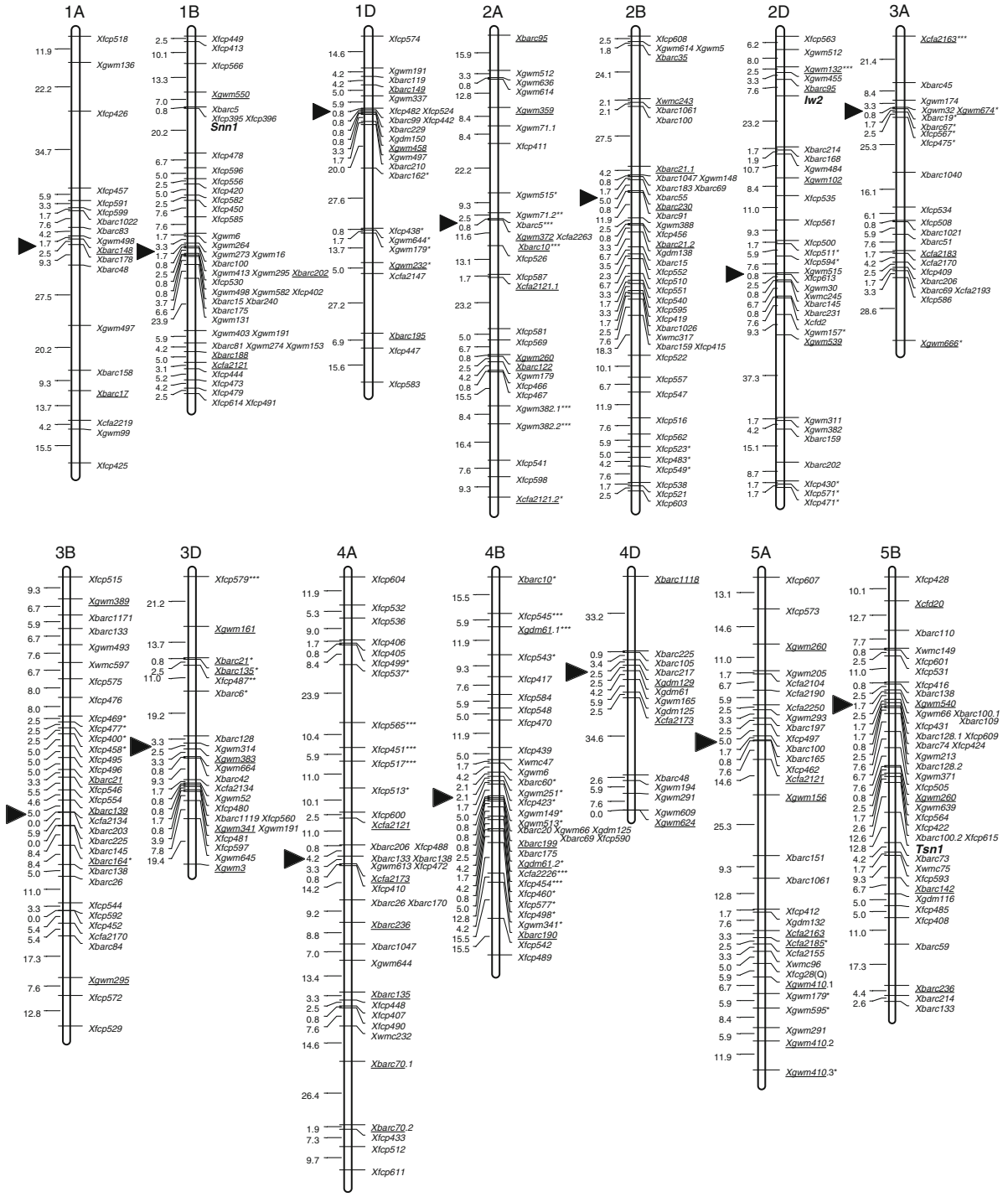

Fig. 1 Genetic linkage maps constructed in the TA4152-60 × ND495 derived DH population. The positions of marker loci are shown to the right of the linkage groups and centiMorgan (cM) distances between loci are shown along the left. Loci in bold font are phenotypic markers. Black triangles indicated the positions of centromeres estimated according to the published physical map (Sourdille et al. 2004). Markers with significantly skewed segregation ratios are indicated with asterisks where *, **, and *** indicates the marker segregation distortion was significant at $P < 0.05, 0.01$ and 0.001, respectively. Underlined markers were assigned to chromosomes using the Chinese Spring (CS) nullisomic-tetrasomic (NT) lines

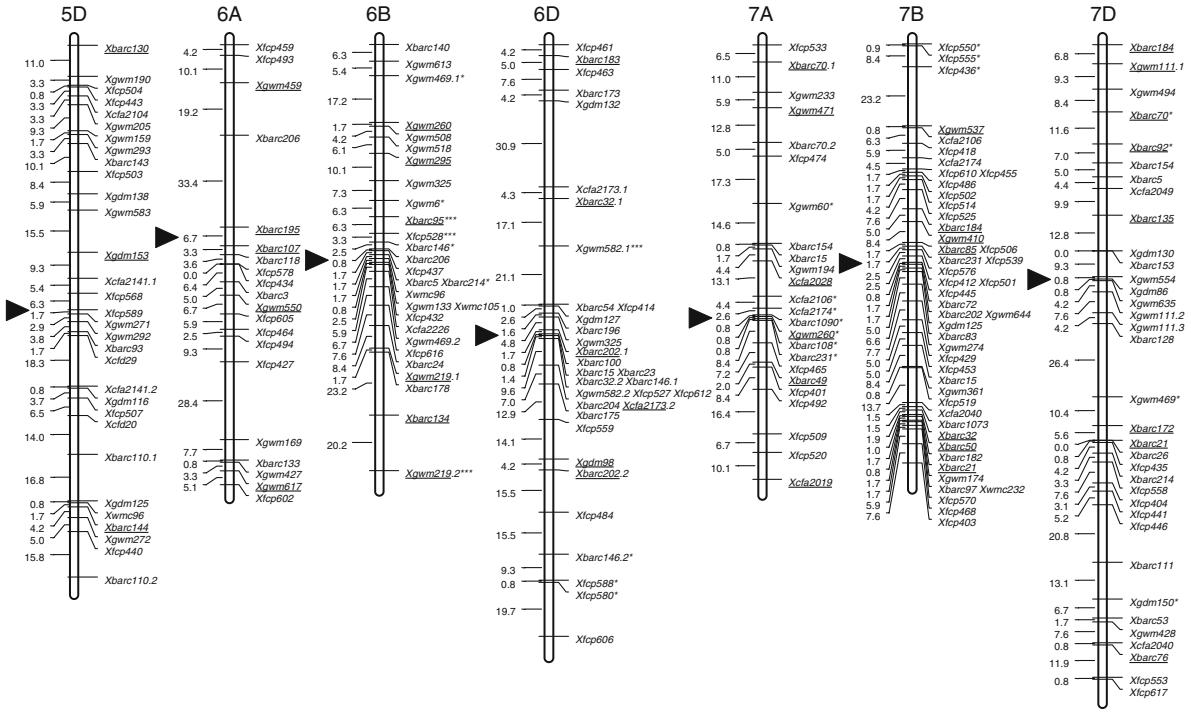

Fig. 1 continued

(Table 2). Marker distribution among the genomes was not uniform. The B genome was more dense than the A and D genomes, and contained 260 markers with an average of 4.8 cM per marker. The A and D genomes had 177 and 189 markers, which gave densities of 7.5 and 6.6 cM per marker, respectively. Almost the same number of SSRs were mapped in the B and D genomes (152 and 142, respectively), whereas fewer SSRs were detected on A genome chromosomes (114). About half (108) of the 218 TRAP markers mapped to the B genome, whereas the A and D genomes possessed only 63 and 47 TRAP markers, respectively. Of the 632 markers, 94 (14.9%) had segregation ratios that deviated significantly from the expected 1:1 ratio. Clusters of markers with skewed segregation ratios were observed on several chromosomes, but chromosome 4B harbored the most (12) markers with distorted segregation ratios (Fig. 1).

The map lengths of individual chromosomes ranged from 105.8 (chromosome 4D) to 247.7 cM (chromosome 4A) (Fig. 1, Table 2). The number of markers per chromosome ranged from 14 on chromosome 4D to 46 on 7B (Table 2), and five chromosomes (1A, 2D, 4D, 6A and 6D) had gaps of more than 30 cM (Fig. 1). Clustering of markers was observed near centromeric regions, particularly for chromosomes 1B, 1D, 4B, 4D, 5B, 6B, 6D, 7A and 7B. However, clustering was also observed in other non-centromeric regions such as 1DL, 2AL, 2BL, 3AL, 3DL, 4BL, 5AL, 5BL, 5DS, 5DL, 7BL and 7DL. The orders and distances of SSR markers on our maps agreed well with those of previously published maps. For the most part, TRAP markers were distributed randomly along each chromosome, but no TRAPs mapped to chromosome 4D, and chromosomes 2B, 4A and 7B contained the most TRAPs (20, 21 and 22, respectively).

Seventeen TRAP markers were generated from the primer combinations involving telomere sequence-based fixed primers, and they were designated *Xfcp601–Xfcp617*. Among these 17 markers, nine mapped to the ends of linkage groups (*Xfcp614*-1BL, *Xfcp603*-2BL, *Xfcp608*-2BS, *Xfcp604*-4AS, *Xfcp611*-4AL, *Xfcp607*-5AS, *Xfcp602*-6AL, *Xfcp606*-6DL and *Xfcp617*-7DL); four mapped to centromeric regions (*Xfcp613*-2D, *Xfcp609*-5B, *Xfcp616*-6B and *Xfcp612*-6D), and the remaining four mapped to other regions (*Xfcp601*-5B, *Xfcp615*-5B, *Xfcp605*-6A and *Xfcp610*-7B) (Fig. 1). Therefore, the nine markers that mapped to ends of linkage groups probably represent the telomeric regions of the chromosomes. Telomere

Table 2 Chromosome assignment and marker distribution, genetic length and marker density of the linkage maps constructed in TA4152-60 × ND495 DH population

Chromosome	Marker				Length (cM)	Marker density (cM/marker)
	SSRs	TRAPs	Other	Total		
1A	12	6	0	18	195.4	10.9
1B	25	17	1	43	166.7	3.9
1D	16	7	0	23	160.6	7.0
2A	19	9	0	28	211.2	7.5
2B	21	20	0	41	211.9	5.2
2D	20	10	1	31	207.9	6.7
3A	16	6	0	22	141.9	6.5
3B	17	16	0	33	190.3	5.8
3D	16	6	0	22	124.5	5.7
4A	15	21	0	36	247.7	6.9
4B	19	15	0	34	163.6	4.8
4D	14	0	0	14	105.8	7.6
5A	24	5	1	30	206.5	6.9
5B	24	14	1	39	180.8	4.6
5D	24	7	0	31	194.6	6.3
6A	11	9	0	20	161.6	8.1
6B	24	4	0	28	159.6	5.7
6D	24	10	0	34	216.6	6.4
7A	17	7	0	24	161.7	6.7
7B	24	22	0	46	170.4	3.7
7D	28	7	0	35	232.1	6.6
A genome	114	63	1	178	1,326.0	7.4
B genome	154	108	2	264	1,243.3	4.7
D genome	142	47	1	190	1,242.1	6.5
Total	410	218	4	632	3,811.4	6.03

sequence-derived TRAP markers mapped to both ends of the chromosome 2B and 4A linkage groups suggesting that these two linkage maps probably span the entire chromosome.

Three phenotypic markers were also mapped (Fig. 1). The DH population segregated in a ratio of 52 glaucous: 68 non-glaucous, which fit the expected 1:1 segregation ratio ($\chi^2_{df=1} = 2.43$, $P = 0.12$). The locus mapped to the distal end of 2DS and the closest marker was *Xbarc95* at 7.6 cM (Fig. 1). This indicates that the glaucousness gene *W1* located on chromosome 2BS was not segregating in this population, but the dominant inhibitor allele at the *Iw2* locus, which had been located on 2DS (Liu et al. 2007), is present in TA4152-60.

ND495 is sensitive to Ptr ToxA and thus carries the dominant *Tsn1* allele, whereas the SHW line is insensitive and carries the recessive *tsn1* allele. The population segregated in a ratio of 57 insensitive: 63 sensitive, which conformed to the expected 1:1 ratio ($\chi^2_{df=1} = 0.3$, $P = 0.58$). The *Tsn1* locus mapped to the long arm of chromosome 5B (Fig. 1) in a position agreeing with that reported by Faris et al. (1996). For reaction to SnTox1, the SHW parent was sensitive and ND495 was insensitive, indicating the former carries the dominant *Snn1* allele. The DH population segregated in a ratio of 52 insensitive: 68 sensitive, which fit the expected 1:1 segregation ratio ($\chi^2_{df=1} = 2.13$, $P = 0.14$). The *Snn1* locus mapped to chromosome arm 1BS and co-segregated with the SSR markers *Xfcp395 and Xfcp396* (Fig. 1).

QTL analysis

A subset of 449 markers spaced approximately 5–20 cM apart and giving the most complete genome

coverage was used for detecting QTLs associated with the agronomic traits. Ear emergence time of TA4152-60 was about 20 days and 14 days later than ND495 in the greenhouse or growth chamber experiments and in the field experiment, respectively. Days to heading among DH lines ranged from 37 to 72 days in the greenhouse and 40–65 days in the field (Fig. 2). Data from greenhouse experiment 1 was homogeneous with the growth chamber experiment (homogeneous group 1), and greenhouse experiment 2 was homogeneous with the field experiment (homogeneous group 2). Therefore, data were combined within the homogeneous groups for QTL analysis. Even though heterogeneity existed between the two homogeneous groups, the trait distribution patterns for both groups were similar. Homogeneous group 1 had peaks at about 47 and 69 days, and homogeneous group 2 had peaks at about 50 and 62 days (Fig. 2).

Two major QTLs (designated as *QEet.fcu-5A* and *QEet.fcu-5B*) associated with days to heading were detected in both homogeneous groups (Fig. 3, Table 3). *QEet.fcu-5A* was located on chromosome 5AL peaking within the *Xgdm132–Xcfa2155* marker interval and explaining 41% of phenotypic variation in homogeneous group 1 and 32% in homogeneous group 2. *QEet.fcu-5B* was located on chromosome 5BL between *Xbarc73* and *Xfcp593*, and it explained 15% of phenotypic variation in homogeneous group 1 and 19% in homogeneous group 2. Earliness was contributed by ND495 for both QTLs, and multiple regression analysis indicated that together, they explained 40–50% of the total phenotypic variation.

TA4152-60 had heights of 120 cm in the greenhouse and 76 cm in the field and was about 38 and 15 cm taller than ND495, respectively. Plant height of DH lines varied from 53 to 136 cm in the greenhouse and 45 to 87 cm in the field (Fig. 2). Comparison of field and greenhouse data indicated they were heterogeneous, but they were homogeneous within greenhouse and field experiments. Therefore, combined greenhouse data and combined field data were analyzed separately. Analysis of the greenhouse data revealed two QTLs. *QHt.fcu-4D* was on chromosome arm 4DS, peaked near *Xbarc225*, and explained 39% of the variation (Fig. 3, Table 3). The second QTL, *QHt.fcu-5A*, was within the *Xfcp412–Xcfa2155* marker interval of the long arm of chromosome 5A, and explained 8% of the phenotypic variation. The chromosomal position of *QHt.fcu-5A* coincided with that of *QEet.fcu-5A*, the QTL associated with days to heading (Fig. 3). For both QTLs, reduced plant height was contributed by ND495, and multiple regression showed that together they explained 40% of the phenotypic variation.

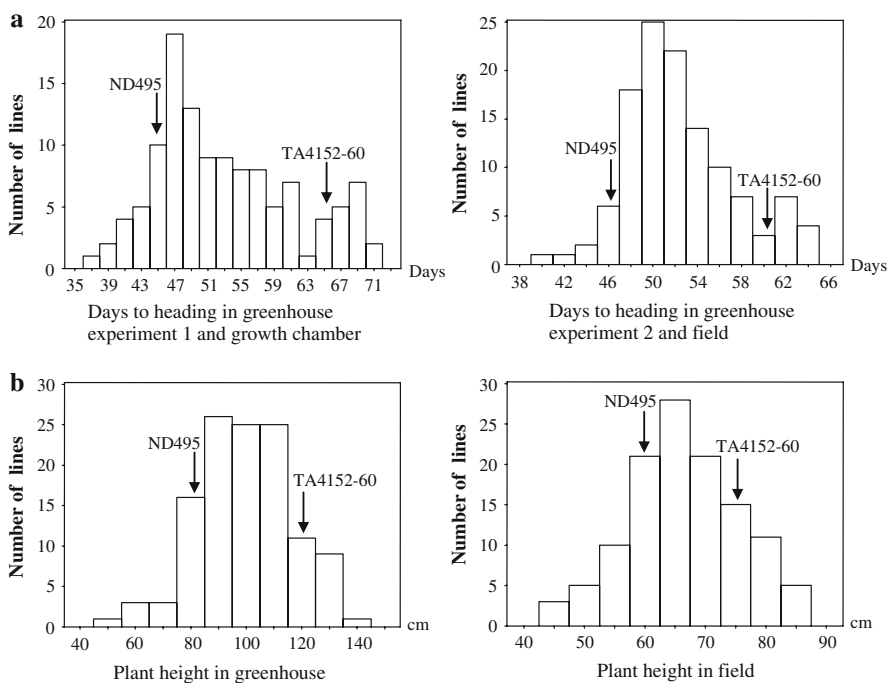

Fig. 2 Histograms of the DH population distribution for the traits of days to heading (**a**) and plant height (**b**)

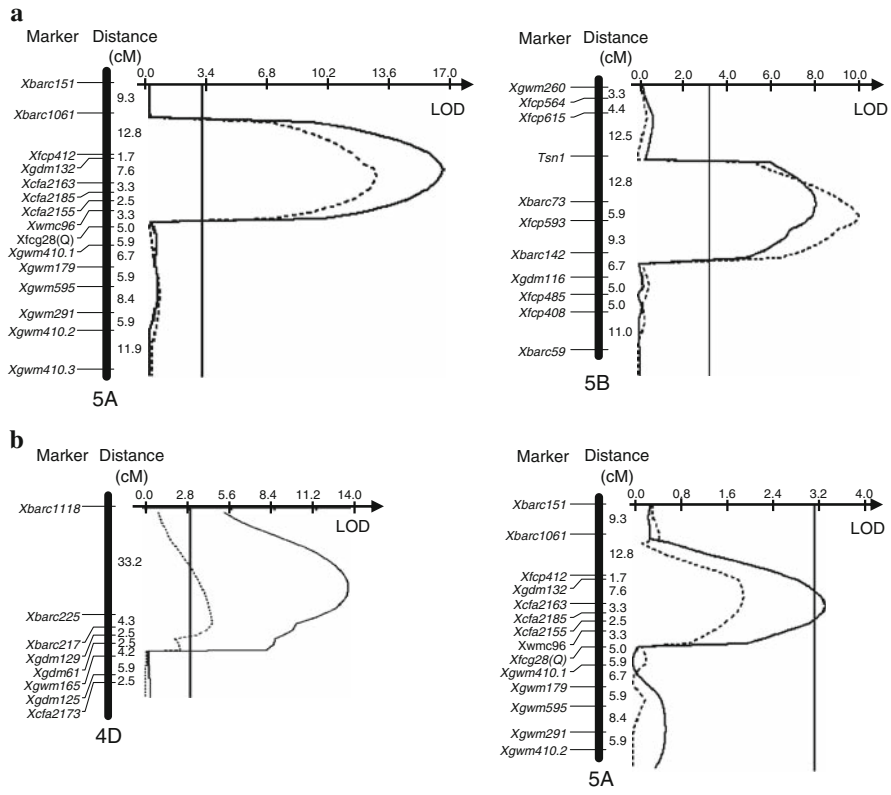

Fig. 3 Composite interval mapping (CIM) of QTLs (LOD > 3) associated with days to heading and plant height in the DH population derived from TA4152-60 × ND495 (**a**) CIM of chromosomes 5A and 5B showing QTLs associated with effects for earliness contributed by ND495. The solid line indicates QTL analysis of greenhouse experiment 1 and growth chamber data combined and the dotted line indicates QTL analysis of greenhouse experiment 2 and field data combined (**b**) CIM of chromosomes 4D and 5A showing QTLs associated with effects for reducing height contributed by ND495. The solid line indicates QTL analysis of greenhouse data and the dotted line indicates QTL analysis of field data

QHt.fcu-4D was also significantly associated with reduced plant height in the field, and explained 14% of the phenotypic variation. No other QTL, including *QHt.fcu*-5A, was found to be significantly associated with plant height in the field (Fig. 3).

Spike-related characteristics were quite different between TA4152-60 and ND495. TA4152-60 had an average spike length of 9 cm with 15 spikelets per spike in the greenhouse and 8 cm with 12 spikelets per head in the field, which yielded a compactness value of 1.6 in the greenhouse and 1.5 in the field. The average spike length for ND495 was 7 cm both in the greenhouse and in the field, and the number of spikelets per spike was 17.6 in the greenhouse and 15 in the field, which resulted in spike compactness values of 2.5 and 2.1, respectively. Among DH lines, spike length varied from 5 to 11 and from 5.5 to 10 cm, spikelet number per spike ranged from 12 to 22 and 10 to 18, and spike compactness values ranged from 1.6 to 3.3 and 1.3 to 2.5, in the greenhouse and in the field, respectively (Fig. 4). The data for spike characteristics were heterogeneous between the greenhouse and field experiments, but there was homogeneity within greenhouse and field experiments and thus combined accordingly.

From both greenhouse and field data, two genomic regions located on chromosomes 3D and 4A were associated with reduced spike length contributed by ND495 and TA4152-60, respectively. The QTLs designated *QEl.fcu-3D* and *QEl.fcu-4A* explained 9% and 14% of phenotypic variation in the greenhouse and 9% and 11% in the field, respectively (Fig. 5, Table 3). Multiple regression indicated that together they explained 15–18% of the variation for spike length. A third QTL associated with reduced spike length contributed by ND495 was detected on chromosome 5A and designated as *QEl.fcu-5A*. This QTL was significant only for the greenhouse experiment where it explained 19% of phenotypic variation and peaked at *Xfcg28(Q)*, which represents the *Q* locus (Fig. 5, Table 3). Together, *QEl.fcu-3D*, *QEl.fcu-4A*, and *QEl.fcu-5A* explained 34.2% of the variation in spike length for the greenhouse data.

A major QTL on chromosome 4DL designated as *QSpn.fcu-4D* and peaking between *Xbarc48* and *Xgwm194*, was associated with increased spikelet

Table 3 Composite interval mapping detected QTLs (LOD > 3) for associated with days to heading, plant height, spike length, spikelet number per spike, and spike compactness in the DH population derived from TA4152-60 × ND495

QTL	Chr[a]	Experiment[b]	Marker interval[c]	Additive effect[d]	LOD	R^2
Days to heading						
QEet.fcu-5A	5AL	GH (Exp 1), GC	*Xgdm132–Xcfa2155*	−8.0 days N	16.59	0.41
		GH (Exp 2), F	*Xgdm132–Xcfa2155*	−4.0 days N	12.71	0.32
QEet.fcu-5B	5BL	GH (Exp 1), GC	*Xbarc73–Xfcp593*	−5.4 days N	7.47	0.15
		GH (Exp 2), F	*Xbarc73–Xfcp593*	−3.3 days N	9.20	0.19
Plant height						
QHt.fcu-4D	4DS	GH	*Xbarc1118–Xbarc225*	−17.6 cm N	13.42	0.39
		F	*Xbarc225–Xgdm61*	−6.1 cm N	4.46	0.14
QHt.fcu-5A	5AL	GH	*Xfcp412–Xcfa2155*	−7.2 cm N	3.17	0.08
Spike length						
QEl.fcu-3D	3DS	GH, F	*Xbarc21–Xfcp487*	−0.45 cm N	3.38	0.09
QEl.fcu-4A	4AL	GH	*Xbarc26–Xbarc236*	−0.65 cm T	6.81	0.14
		F	*Xbarc26–Xbarc236*	−0.43 cm T	3.97	0.11
QEl.fcu-5A	5AL	GH	*Xwmc96–Xgwm410.1*	−0.87 cm N	8.50	0.19
Spikelet number per head						
QSpn.fcu-4D	4DL	GH	*Xbarc48–Xgwm194*	1.7 T	8.13	0.29
		F	*Xbarc48–Xgwm194*	1.1 T	3.25	0.15
Spike compactness						
QEc.fcu-5A	5AL	GH	*Xfcg28–Xgwm179*	0.16 N	5.40	0.08
QEc.fcu-5B	5BL	GH	*Xfcp593–Xbarc142*	0.22 T	5.09	0.13
		F	*Xfcp593–Xbarc142*	0.16 T	6.98	0.11

[a] Chr = Chromosome

[b] GH = greenhouse; GC = growth chamber; F = field; Exp = experiment

[c] The markers delineate the 95% confidence interval for the QTL

[d] Additive indicates additive main effect contributed by allele from corresponding parent, where N = ND495 and T = TA4152-60. A negative indicates the QTL allele showed a reduced effect

number per spike contributed by TA4152-60. *QSpn.fcu-4D* was significant for both the greenhouse and field data, and explained 29% and 15% of phenotypic variation, respectively (Fig. 5, Table 3).

For spike compactness, *QEc.fcu-5B* associated with increasing compactness contributed by TA4152-60 on chromosome arm 5BL was detected from both greenhouse and field data, and it explained 13% and 11% of phenotypic variation, respectively (Fig. 5, Table 3). *QEc.fcu-5A* associated with increased spike compactness contributed by ND495 was detected using the greenhouse data but not the field data. This QTL peaked at *Xfcg28(Q)* on 5AL and explained 8% of the phenotypic variation (Fig. 5, Table 3). *QEc.fcu-5B* and *QEc.fcu-5A* together explained 15.4% of the variation in spike compactness for the greenhouse experiments.

Discussion

The SHW line TA4152-60 showed high levels of resistance to tan spot and SNB (Xu et al. 2004), STB, FHB, leaf rust, and the insect pest Hessian fly (unpublished data). ND495 is susceptible to all of the diseases and pest mentioned, but it is superior to TA4152-60 for numerous agronomic traits including seed dormancy, plant height, ear emergence time, maturity, threshability, etc. Therefore, a large amount of variation exists in this DH population, which makes it a useful tool for identifying QTLs associated with disease and pest resistance as well as important agronomic traits. Because experiments using modest numbers of individuals (100–200) were shown to be sufficient for detecting most major QTLs (Lynch and Walsh 1989), a subset of 120 DH lines from our

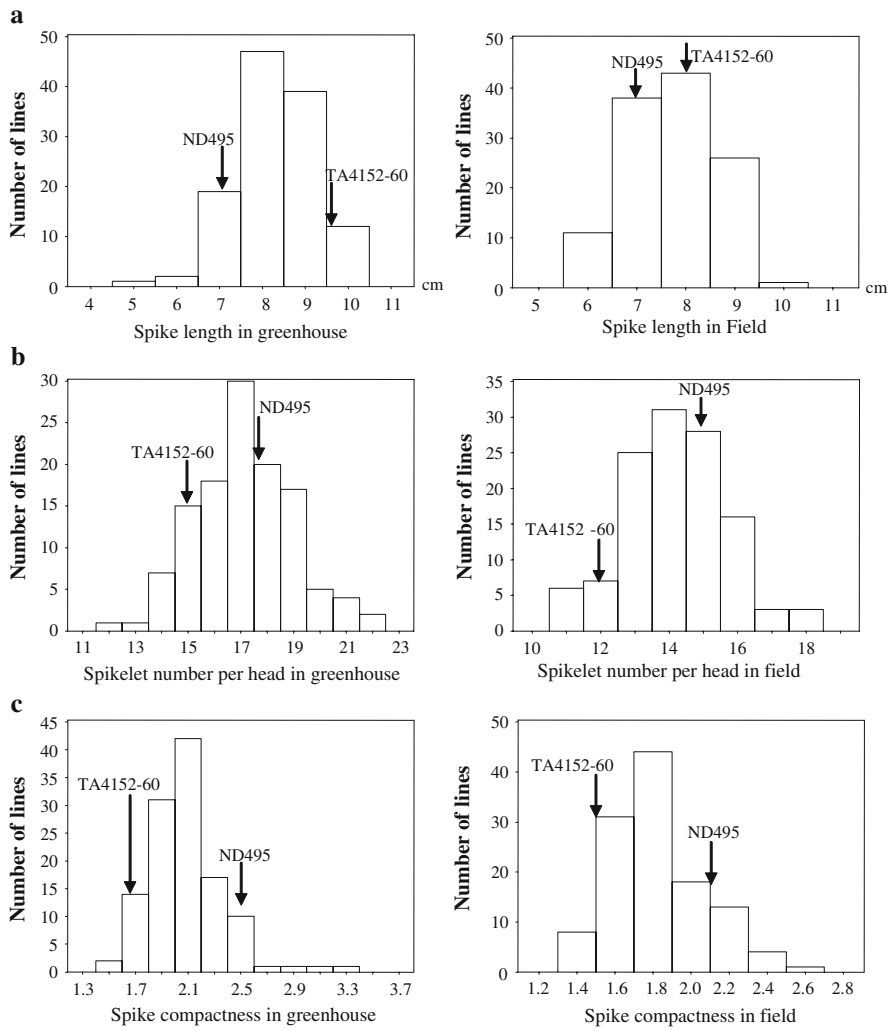

Fig. 4 Histograms of the DH population distribution for spike related traits (**a**) spike length, (**b**) spikelet number per spike, and (**c**) spike compactness

population was randomly selected and used for mapping and QTL analysis. However, the population consists of 213 DH lines, and the use of the entire population would likely increase the power to detect QTLs with relatively minor effects as well.

The genetic lengths of published hexaploid wheat maps ranged from 2,360 to 4,110 cM (Chalmers et al. 2001; Groos et al. 2002; Liu et al. 2005; Paillard et al. 2003; Quarrie et al. 2005; Somers et al. 2004; Song et al. 2005; Sourdille et al. 2003; Torada et al. 2006). The total length of our maps was 3,811.5 cM, which is slightly larger than most of the published maps, but smaller than the 4,110 cM reported by Chalmers et al. (2001). The largest linkage groups in our maps were for chromosomes 4A (247.7 cM) and 7D (232.1 cM), and the shortest were chromosomes 4D (105.5 cM) and 3D (124.5 cM). Our results are in agreement with most previously published maps in that chromosome 4D is one of the shortest linkage groups and chromosome 7D is one of the longest, but our 3D map is shorter relative to others (Guyomarc'h et al. 2002; Quarrie et al. 2005; Somers et al. 2004; Sourdille et al. 2003). This may be due to a lack of polymorphism on chromosome 3D in our population. The size of our chromosome 4A map is relatively larger than other published maps of 4A. This may be due to the telomere sequence-derived TRAP markers that mapped to both ends of our 4A linkage group, which indicates that our 4A linkage group probably represents the entire chromosome.

Assuming that the genetic length of the entire hexaploid wheat genome is about 4,000 cM, our

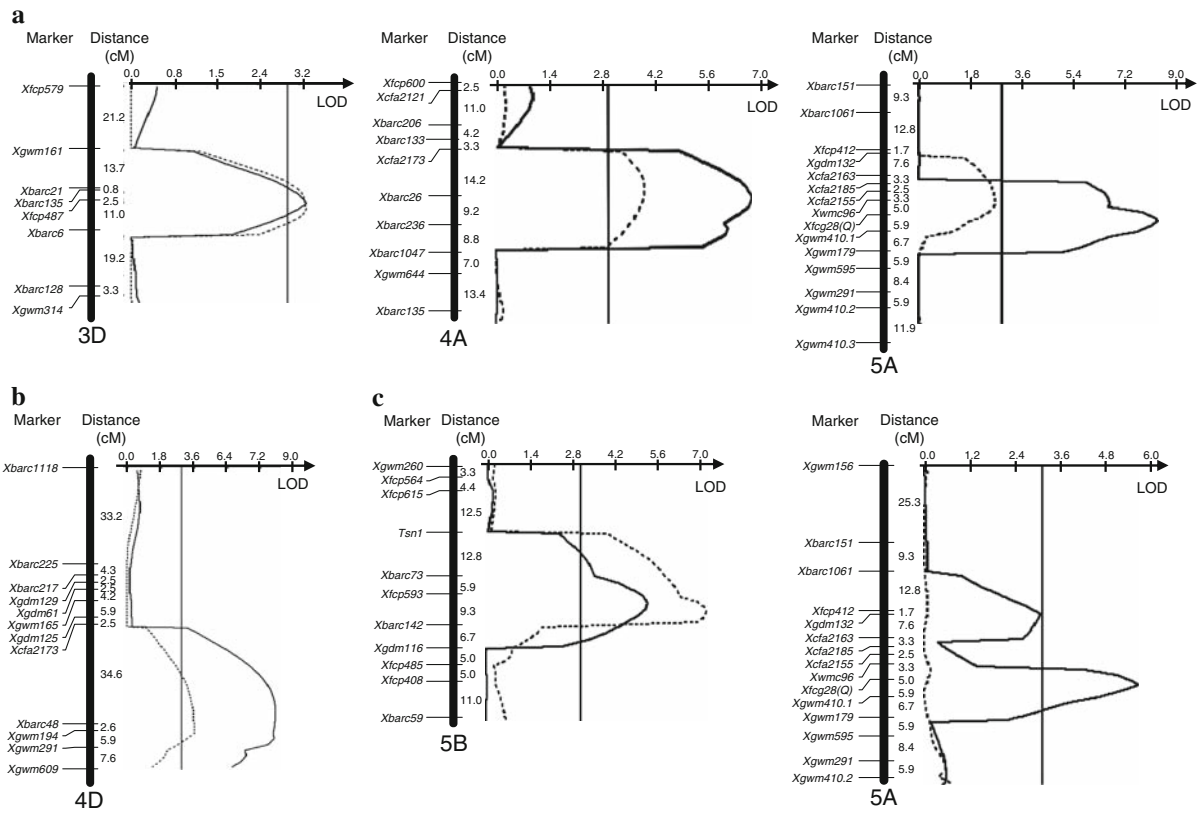

Fig. 5 Composite interval mapping (CIM) of QTLs (LOD > 3) associated with spike related characteristics in the DH population derived from TA4152-60 × ND495. The solid line indicates QTL analysis of greenhouse data and the dotted line indicates QTL analysis of field data (**a**) CIM of chromosomes 3D and 5A showing QTLs associated with effects for reducing spike length contributed by ND495, and 4A showing QTLs associated with reduced length contributed by TA4152-60 (**b**) CIM of chromosome 4D showing the QTL associated with effects for increasing spikelet number per spike contributed by TA4152-60 (**c**) CIM of chromosomes 5A and 5B showing major QTLs associated with effects for increasing spike compactness contributed by ND495 and TA4152-60, respectively

maps have about 95% coverage. The relatively high genome coverage of our map was in part due to the telomere sequence-based TRAP markers that were used to define the ends of several linkage groups. Nine out of 17 telomere sequence-based TRAP markers mapped to chromosome ends, and both ends of chromosomes 2B and 4A were capped by these markers (Fig. 1). Therefore, our results also showed telomere sequence-derived fixed primers could generate chromosome end-specific TRAP markers.

Uniformity of marker distribution is also important for detecting all possible loci associated with a trait of interest. Most previously published maps reported the clustering of markers in proximal regions of chromosomes (Liu et al. 2005; Quarrie et al. 2005; Somers et al. 2004; Torada et al. 2006) due to the fact that recombination is highly suppressed in those regions, and they commonly contain gaps of more than 50 cM in distal regions (Liu et al. 2005; Paillard et al. 2003; Quarrie et al. 2005; Sourdille et al. 2003; Torada et al. 2006) due to a high frequency of recombination, lack of polymorphism, or both. Our maps also showed uneven distribution of markers, but they contained no gaps greater than 50 cM and only had six gaps >30 cM. This was primarily due to the efficiency of the TRAP marker technique. Not only did the use of TRAPs increase marker density, but it also allowed us to fill gaps between SSR marker loci and, as mentioned above, to extend linkage groups into the telomeric regions in a number of instances providing more complete genome coverage.

Segregation distortion of markers is a common phenomenon that has been observed on previously published maps and the percentage of distorted

markers ranged from 3 (Quarrie et al. 2005) to 27% (Cadalen et al. 1998). Chalmers et al. (2001), Liu et al. (2005) and Quarrie et al. (2005) all reported chromosome 4A as harboring the most markers with skewed segregation ratios. We observed segregation distortion on numerous chromosomes (Fig. 1), but chromosome 4B carried the most markers with distorted ratios, which were mostly clustered in the centromeric region (Fig. 1). After checking the marker genotypes of each DH line in this region, we found that distortion was skewed in favor of TA4152-60 alleles. It is possible that chromosome 4B of the SHW line harbors gene(s) that cause competition among female gametes for preferential fertilization by maize pollen, or that increase the viability of embryos during the rescuing process.

QTL analysis demonstrated the utility of our maps for identifying major QTLs associated with agronomic traits. For days to heading, two major QTLs associated with effects for earliness contributed by ND495 were identified on 5AL and 5BL (Fig. 3). Kato et al. (1999) reported two QTLs on 5A associated with ear emergence time. One QTL was in the vicinity of *Vrn-A1*, while the other was near the known location of the *Q* gene. *QEet.fcu-5A* may be the same as the first QTL reported by Kato et al. (1999) because *QEet.fcu-5A* was not associated with the *Q* locus but was located in the *Vrn-A1* region. Therefore, *QEet.fcu-5A* probably corresponds to *Vrn-A1*, which was also indicated by Hanocq et al. (2004). The second QTL for days to heading identified in this research, *QEet.fcu-5B*, may correspond to *Vrn-B1*. The closest marker to this QTL on our map was *Xwmc75*, which is in the vicinity of *Vrn-B1* based on the linkage maps of Tóth et al. (2003) and Somers et al. (2004). Therefore, it seems that variation in days to heading in our DH population was likely influenced by allelic variation at vernalization loci. However, it is also possible that other genes within the vicinity of the vernalization loci are involved.

The major QTL associated with plant height was *QHt.fcu-4D* (Fig. 3), and its position coincided with *Rht-D1* (Ellis et al. 2002), indicating different alleles of *Rht-D1* are carried by ND495 and TA4152-60. The minor QTL, *QHt.fcu-5A*, associated with plant height was only detected using the greenhouse data and was located in the vicinity of *Vrn-A1* (Fig. 3). Kato et al. (1999) reported the *Vrn-A1* locus to have a height-reducing effect, which suggests that *Vrn-A1* may be a candidate for *QHt.fcu-5A*.

Several genomic regions associated with ear morphology were detected in this DH population (Fig. 5). *QEl.fcu-4A* associated with effects for reducing spike length contributed by TA4152-60 was identified from both greenhouse and field experiments and it may be the same as a QTL reported by Sourdille et al. (2000). *Xbarc206* was the closest marker to *QEl.fcu-4A* on our map, and *Xwmc173* was the closest marker to the 4A QTL reported by Sourdille et al. (2000). *Xwmc173* was not mapped in our population and *Xbarc206* is not present on the 4A map of Sourdille et al. (2000), but the two markers lie 5 cM apart on the 4A map of Somers et al. (2004).

The *Q* locus defined the peak of the spike length QTL, *QEl.fcu-5A*, which was significant for greenhouse experiments. *Q* is a member of the *AP2*-family of plant transcription factors (Simons et al. 2006) and is considered to be a major wheat domestication gene with pleiotropic effects. Traits influenced by the *Q* locus include the free-threshing (naked grain) character, plant height, days to heading, rachis fragility, glume tenacity, and spike morphology including spike length (Muramatsu 1986; Kato et al. 1999; Faris et al. 2003; Simons et al. 2006). Therefore, it would seem likely that *Q* is responsible for the effects of *QEl.fcu-5A* in this population. However, all domesticated durum and common wheat cultivars are considered to possess the same *Q* allele on chromosome 5A that governs these characters (Simons et al. 2006), and we would not expect there to be allelic variation at the *Q* locus between ND495 and the SHW parent, which obtained its 5A *Q* allele from the durum variety Scoop 1. Sequencing and expression analysis of the parental *Q* alleles is needed to shed light on this discrepancy. The minor QTL, *QEl.fcu-3D*, associated with reduced spike length contributed by ND495 was detected in all experiments. To our knowledge, there have been no previous reports of spike length QTLs on chromosome 3D. Therefore, this QTL represents a novel locus affecting spike length in our DH population.

Major QTLs on chromosome arms 4DL (*QSpn.fcu-4D*) and 5BL (*QEc.fcu-5B*) detected from all experiments were found associated with spikelet number per spike and spike compactness, respectively. There are no obvious candidate genes that underlie these QTLs, but it is possible that

QEc.fcu-5B might represent the effect of the homoeologous *q* allele on chromosome 5B. The precise mapping of the 5B *q* allele is needed to determine if this is indeed the case. Another QTL (*QEc.fcu-5A*) associated with effect of increasing spike compactness in the greenhouse contributed by ND495 was located on chromosome arm 5AL and peaked at the *Q* locus. The *Q* locus is known to affect spike compactness (Muramatsu 1986), but as discussed above, we would not expect allelic variation to exist between the parents of our population and further experiments are needed to resolve this issue.

In conclusion, we used the wheat × maize method to develop a DH population and a combination of SSRs and TRAPs to develop a 632-locus whole genome map in about 3 years. We demonstrated the utility of the maps for QTL analysis by identifying novel and known QTLs associated with various agronomic traits. Therefore, the DH population and genetic map will be a useful tool for the identification of disease resistance QTL and other agronomically important loci, and will aid in the identification and development of markers for marker-assisted selection (MAS).

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